High prevalence of Ranavirus infection in permanent constructed wetlands in Eastern Kentucky, USA

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Abstract:
Two important drivers of global amphibian declines are land-use change and infectious diseases. Hundreds of permanent wetlands have been constructed on ridge tops for wildlife management in eastern Kentucky within the same landscape as natural, ephemeral wetlands. Constructed wetlands support a different amphibian community than natural wetlands, and some of these species have been associated with ranavirus outbreaks. Our objective was to test for occurrence of ranavirus and amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibian populations of natural and constructed wetlands in the Daniel Boone National Forest, Kentucky. We selected Eastern Newts (*Notopthalmus viridescens*) and Wood Frogs (*Lithobates sylvaticus*) as representative species of constructed and natural wetland amphibian communities. Samples taken from 10 adult newts from five constructed wetlands and 10 Wood Frog larvae from one natural wetland in May 2012 were tested to determine the presence of ranavirus and chytrid fungus. No *Batrachochytrium dendrobatidis* was detected, but 9 samples from two constructed wetlands were positive for ranavirus (prevalence = 70% and 33%). Adult newts are known to move among wetlands in close proximity including permanent and ephemeral wetlands and are a potential reservoir species for transmitting ranavirus to natural wetlands.
Introduction:

- Land-use change and infectious diseases are major drivers of global amphibian decline (Miller et al. 2011; Stuart et al. 2004)
- Hundreds of closely spaced permanent wetlands have been constructed on ridge tops in eastern Kentucky for wildlife management within the same landscape as natural, ephemeral wetlands (Brown and Richter 2012; Figure 1)

Constructed wetlands might not replace the function of natural wetlands, supporting different amphibian communities than natural ponds and have been associated with ranavirus outbreaks (Denton and Richter 2013

Methods:

- Field surveys were conducted in five constructed and one natural ridge-top wetland located in the Daniel Boone National Forest (DBNF), Kentucky
- Dipnet sampling was used to capture up to 10 adult Eastern Newts (*Notopthalmus viridescens*) in each constructed wetland and 10 Wood Frog (*Lithobates sylvaticus*) larvae in the natural wetland (Figure 2)
- Each Eastern Newt was swabbed using a BBLTM CultureSwabTM and had a 10-mm portion of its tail clipped and each *L. sylvaticus* larva was euthanized in 10% ethanol and stored in 95% ethanol (Figure 3)
- Ranavirus and *Bd* testing was performed at the University of Tennessee Center for Wildlife Health
- Genomic DNA (gDNA) was extracted from a homogenate of liver and kidney tissue (Wood Frogs), tail clips (Eastern Newts), and swabs (Eastern Newts) using a commercially available kit

Positive controls were similar for each assay, and included DNA extracted from culture and a positive animal for each pathogen.

Results:

- No *Batrachochytrium dendrobatidis* was detected
- 9 samples from two constructed wetlands were positive for ranavirus (prevalence = 70% and 33%)

Discussion:

- Adult newts are known to move among wetlands in close proximity (Porej et al. 2004) and use ephemeral and permanent wetlands (Hunsinger and Lanoo 2005) and may transport ranavirus overland among sites into amphibian communities composed of highly susceptible species

- There are several reasons constructed ponds might have important consequences for ranavirus epidemiology
    - Ranavirus virions are inactivated faster in dry soil compared to water, the long hydroperiods might increase the persistence of ranavirus outside the host (Nazir et al. 2012)
    - The absence of *Bd* in the constructed wetlands might be attributed to lack of substrate complexity and shade, or it could be because *Bd* has not arrived to the ecosystem or was simply not detected (Raffel et al. 2010)
    - Constructed wetlands were inhabited by amphibian species that require a longer hydroperiod for development and may function as reservoirs for ranavirus, including Eastern Newts, American Bullfrogs (*L. catesbeianus*), and Green Frogs (*L. clamitans*)

Conclusions:

Batrachochytrium dendrobatidis was not detected, but ranavirus was detected in 9 samples from two constructed wetlands . Previously, ravavirus has been documented in only two wetlands in Kentucky. We recommend more intensive studies in the future that examine a larger geographic area and larger sample size per wetland type.